

CLAIMS

1. A method for preparing a cytotoxic lymphocyte characterized in that the method comprises the step of carrying out at least one of induction, maintenance
5 and expansion of a cytotoxic lymphocyte in the presence of fibronectin, a fragment thereof or a mixture thereof.

2. The method according to claim 1, wherein the cytotoxic lymphocyte highly expresses an interleukin-2 receptor as compared to a cytotoxic
10 lymphocyte obtained by carrying out at least one of induction, maintenance and expansion in the absence of fibronectin, a fragment thereof or a mixture thereof.

3. The method according to claim 1, wherein the cytotoxic lymphocyte contains CD8-positive cell in a higher ratio as compared to a cytotoxic
15 lymphocyte obtained by carrying out at least one of induction, maintenance and expansion in the absence of fibronectin, a fragment thereof or a mixture thereof.

4. The method according to any one of claims 1 to 3, wherein the cytotoxic lymphocyte highly maintains cytotoxic activity as compared to a cytotoxic
20 lymphocyte obtained by carrying out at least one of induction, maintenance and expansion in the absence of fibronectin, a fragment thereof or a mixture thereof.

5. The method according to any one of claims 1 to 4, wherein fibronectin, a fragment thereof or a mixture thereof is immobilized in a solid phase.

6. The method according to claim 5, wherein the solid phase is a cell culture equipment or a cell culture carrier.

7. The method according to claim 6, wherein the cell culture equipment is a petri dish, a flask or a bag, and the cell culture carrier is beads, a membrane or a slide glass.

8. The method according to any one of claims 1 to 4, wherein at least one of induction, maintenance and expansion of a cytotoxic lymphocyte is carried out in a medium containing fibronectin, a fragment thereof or a mixture thereof.

9. The method according to any one of claims 1 to 8, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

10. The method according to claim 9, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

11. The method according to claim 9, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

12. The method according to claim 1 comprising carrying out at least one of induction, maintenance and expansion of a cytotoxic lymphocyte in the presence of fibronectin, a fragment thereof or a mixture thereof in a cell culture equipment containing a medium, wherein the method satisfies any one of the conditions of:

- 5 (a) a ratio of the number of cells at initiation of culture to a culture area in the cell culture equipment being 1 to 5×10^5 cells/cm²; and
- (b) a concentration of cells in a medium at initiation of culture being 1 to 5×10^5 cells/ml.

10 13. The method according to claim 12, wherein the method excludes a dilution step or a step of exchanging a cell culture equipment.

14. A cytotoxic lymphocyte obtained by the method of any one of claims 1 to 13.

15 15. A medicament comprising as an effective ingredient a cytotoxic lymphocyte obtained by the method of any one of claims 1 to 13.

16. An agent for enhancing an interleukin-2 receptor expression of a cell,
20 characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

17. The agent according to claim 16, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by
25 SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution,

deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

5 18. The agent according to claim 17, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

10 19. The agent according to claim 17, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

20. An agent for improving a ratio of CD8-positive cell in a lymphocyte, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

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21. The agent according to claim 20, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid
20 sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

22. The agent according to claim 21, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

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23. The agent according to claim 21, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

5 24. An agent for improving or maintaining cytotoxic activity in a cytotoxic lymphocyte, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

10 25. The agent according to claim 24, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

15 26. The agent according to claim 25, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

20 27. The agent according to claim 25, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

25 28. A method for increasing expression of an interleukin-2 receptor in a cytotoxic lymphocyte, characterized in that the method comprises the step of carrying out at least one of induction, maintenance and expansion of a cytotoxic

lymphocyte in the presence of fibronectin, a fragment thereof or a mixture thereof.

29. A method for improving a ratio of CD8-positive cell in a cytotoxic lymphocyte, characterized in that the method comprises the step of carrying out at least one of induction, maintenance and expansion of a cytotoxic lymphocyte in the presence of fibronectin, a fragment thereof or a mixture thereof.

30. A method for improving or maintaining cytotoxic activity in a cytotoxic lymphocyte, characterized in that the method comprises the step of carrying out at least one of induction, maintenance and expansion of a cytotoxic lymphocyte in the presence of fibronectin, a fragment thereof or a mixture thereof.

31. The method according to any one of claims 1 to 13, further comprising the step of transducing a foreign gene into a cytotoxic lymphocyte.

32. The method according to claim 31, wherein the foreign gene is transduced using retrovirus, adenovirus, adeno-associated virus or simian virus.